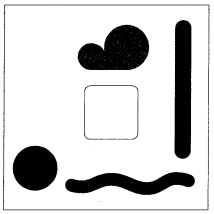

RCC - CCR PROJECT 605900

SALMONELLA TYPHIMURIUM REVERSE MUTATION ASSAY

WITH

Wacker BS 1701

REPORT

Study Completion Date:
May 12, 1998

RCC

COPY OF GLP CERTIFICATE

HESSISCHES MINISTERIUM FÜR
UMWELT, ENERGIE, JUGEND,
FAMILIE UND GESUNDHEIT

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung(en)
CCR Cytotest Cell Research GmbH & Co. KG
in 64380 Roßdorf, In den Leppsteinswiesen19
(Ort, Anschrift)
der RCC/CCR Holding Verwaltungs GmbH
(Firma)
am 05./06./07. April 1995
(Datum)

von der für die Überwachung zuständigen Behörden über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Certificate

It is hereby certified that the test facility(ies)
CCR Cytotest Cell Research GmbH & Co. KG
in 64380 Roßdorf, In den Leppsteinswiesen 19
(location, address)
of RCC/CCR Holding Verwaltungs GmbH
(company name)
on 05./06./07. April 1995
(date)

was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Prüfkategorie nach § 19 d Abs. 3 Chemikaliengesetz in der Fassung vom 29.Juli 1994 (BGBl. I S. 1703), zuletzt geändert am 27. September 1994 (BGBl. I S. 2705) in Verbindung mit der Allgemeinen Verwaltungsvorschrift zum Verfahren der behördlichen Überwachung der Einhaltung der Grundsätze der Guten Laborpraxis vom 21. Oktober 1990 (BAnz. 204 a vom 31.10.1990):

Toxikologische Eigenschaften	Toxicological properties

Prüfkategorie gemäß OECD Panel on Good Laboratory Practice (January 1992)

Prüfungen auf toxikologische Eigenschaften	Toxicity studies
Prüfungen auf mutagene Eigenschaften (in vitro, in vivo)	Mutagenicity studies

Im Auftrag
Dr. Hecker
(Dr. Hecker) Wiesbaden, den 2. August 1995

HESSISCHES MINISTERIUM FÜR UMWELT, ENERGIE, JUGEND, FAMILIE UND GESUNDHEIT

CONTENTS

PREFACE

General

Sponsor:	Wacker-Chemie GmbH Werk Burghausen Johannes-Hess-Str. 24 D-84489 Burghausen
Study Monitor:	Dr. Axel Bosch
Testing Facility:	**R C C** CYTOTEST CELL RESEARCH GMBH In den Leppsteinswiesen 19 D-64380 Roßdorf
RCC-CCR Project No.:	605900
Test Article:	Wacker BS 1701
RCC-CCR Test Article No.:	S1466 11
Title:	Salmonella typhimurium Reverse Mutation Assay with Wacker BS 1701

Project Staff

Study Director:	Dr. Hans-Eric Wollny
Management:	Markus Arenz
Quality Assurance Unit:	Frauke Hermann

Schedule

Date of Protocol:	March 19, 1998
Date of 1st Amendment to Protocol:	April 17, 1998
Start of Pre-Experiment:	March 24, 1998
End of Pre-Experiment:	March 30, 1998
Start of Experiment I:	March 24, 1998
End of Experiment I:	April 03, 1998
Start of Experiment II:	April 03, 1998
End of Experiment II:	April 06, 1998
Date of Draft:	April 17, 1998
Date of Final Report:	May 12, 1998

Project Staff Signatures

Study Director — Dr. Hans-Eric Wollny

Date: May 12, 1998

Management — Markus Arenz

ppa

Date: May 12, 1998

Quality Assurance

The study was performed in compliance with:

„ Chemikaliengesetz“ (Chemicals Act) of the Federal Republic of Germany, „ Anhang 1“ (Annexe 1) dated July 25, 1994 („BGBl. I 1994“, pp. 1703), last revision: May 14, 1997

"The OECD Principles of Good Laboratory Practice", Paris, 1981.

Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

"Ninth Addendum to OECD Guidelines for Testing of Chemicals", Section 4, No. 471: "Bacterial Reverse Mutation Assay", adopted July 21, 1997

EEC Directive 92/69, L 383 A, Annexe V, B 14, dated December 29, 1992

"Japanese Guidelines for Screening Toxicity Testings of Chemicals:
Testing Methods for New Chemical Substances enacted July 13, 1974, amended December 5, 1986"

Archiving

RCC Cytotest Cell Research GmbH, D-64380 Roßdorf will archive the following data for 15 years:

Raw data, protocol, and a copy of the report.

The following sample will be archived for at least 2 years following the date on which the report is audited by the Quality Assurance Unit and also at least until the next inspection of RCC Cytotest Cell Research by the GLP-authority:

A sample of the test article

If there are no other instructions by the sponsor the raw data and the above mentioned material will be discarded at the end of the archiving period.

Deviations to Protocol

There were no deviations to protocol

STATEMENT OF COMPLIANCE

Project Number:	605900
Test Article:	Wacker BS 1701
Study Director:	Dr. Hans-Eric Wollny
Title:	Salmonella Typhimurium Reverse Mutation Assay with Wacker BS 1701

This study performed in the testing facility of RCC Cytotest Cell Research was conducted in compliance with Good Laboratory Practice Regulations.

„ Chemikaliengesetz“ (Chemicals Act) of the Federal Republic of Germany, „ Anhang 1“ (Annexe 1) dated July 25, 1994 („BGBl. I 1994“, pp. 1703), last revision: May 14, 1997

"The OECD Principles of Good Laboratory Practice", Paris, 1981.

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

R C C - C C R
Dr. Hans-Eric Wollny

Wollny
.....

Date: May 13, 1998

QUALITY ASSURANCE UNIT

RCC Cytotest Cell Research GmbH
In den Leppsteinswiesen 19,
D-64380 Roßdorf

Statement

Project Number:	605900
Test Article:	Wacker BS 1701
Study Director:	Dr. Hans-Eric Wollny
Title:	Salmonella Typhimurium Reverse Mutation Assay with Wacker BS 1701

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates.

Dates and phases of QAU Inspections/ Audits			Dates of Reports to the Study Director and to Management	
Protocol Audit:	March	20, 1998	March	20, 1998
1st Amendment to Protocol Audit:	April	22, 1998		
2nd Amendment to Protocol Audit:	May	13, 1998		
Study Inspection:	April	03, 1998	April	03, 1998
Draft Audit:	April	22, 1998	April	22, 1998

Head of Quality Assurance Unit — Frauke Hermann

F. Hermann

Date: May 13, 1998

SUMMARY OF RESULTS

This study was performed to investigate the potential of Wacker BS 1701 to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, and TA 100, and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate

No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation.

The plates incubated with the test article showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Wacker BS 1701 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

Conclusion

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, Wacker BS 1701 is considered to be non-mutagenic in this Salmonella typhimurium reverse mutation assay.

OBJECTIVE

Aims of the Study

The experiments were performed to assess the potential of the test article to induce gene mutations by means of two independent Salmonella typhimurium reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since a negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

Reasons for the Study

The most widely used assays for detecting gene mutations are those using bacteria. They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

Reverse mutation assays determine the frequency with which an agent reverses or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxotrophy to prototrophy) is the most widely used marker.

The Salmonella typhimurium histidine (his) reversion system measures $his^- \rightarrow his^+$ reversions. The S. typhimurium strains are constructed to differentiate between base pair (TA 1535, TA 100, TA 102) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation and the pre-incubation method the bacteria are exposed to the test article with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect six dose levels with adequately spaced concentrations were tested. The maximum dose level was 5000 µg/plate.

To validate the test, reference mutagens are tested in parallel to the test article.

MATERIALS AND METHODS

Test Article

The test article and the information concerning the test article were provided by the sponsor.

Name:	Wacker BS 1701
Batch No.:	1244KH
Aggregate State at Room Temperature:	liquid
Colour:	colourless
Purity:	> 95 % (2,4,4-Trimethylpentyltriethoxysilane and isomers)
Stability in Solvent:	24 hours in ethanol, acetone, and DMSO
Storage:	room temperature, light protected
Expiration Date:	March, 2000

On the day of the experiment, the test article Wacker BS 1701 was dissolved in DMSO. The solution was neutralised with NaOH. The solvent was chosen because of its solubility properties and its relative nontoxicity to the bacteria.

No precipitation of the test article occurred up to the highest investigated dose.

Controls

Negative Controls

Concurrent untreated and solvent controls were performed.

Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg
Catalogue No.:	30175
Purity:	at least 99 %
Dissolved in:	water deionised
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	N 9504
Purity:	> 99.9 %
Dissolved in:	DMSO
Concentration:	10 µg/plate in TA 98, 50 µg/plate in TA 1537

Strain:	TA 102
Name:	methyl methane sulfonate, MMS
Supplier:	MERCK-SCHUCHARDT, D-85662 Hohenbrunn
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	water deionised
Concentration:	5.0 µl/plate

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, TA 102
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen
Catalogue No.:	A 1381
Purity:	97.5 %
Dissolved in:	DMSO
Concentration:	2.5 µg/plate (10.0 µg/plate in TA 102)

The stability of the positive control substances in solution was unknown but a mutagenic response in the expected range is sufficient evidence of biological stability. The dilutions of the stock solutions were prepared on the day of the experiment and used immediately.

Test System

Characterisation of the Salmonella typhimurium Strains

The histidine dependent strains are derived from S. typhimurium strain LT2 through a mutation in the histidine locus. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes an inactivation of the excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98 and TA 100 and TA 102 the R-factor plasmid pKM 101 carries the ampicillin resistance marker. The strain TA 102 does not contain the $uvrB^-$-mutation and is excision repair proficient. Additionally, TA 102 contains the multicopy plasmid pAQ1 carrying the hisG428 mutation (ochre mutation in the hisG gene) and a tetracycline resistance gene.

In summary, the mutations of the TA strains used in this study can be described as follows:

Salmonella typhimurium

TA1537: his C 3076; rfa^-; $uvrB^-$:	frame shift mutations
TA 98: his D 3052; rfa^-; $uvrB^-$;R-factor:	" " "
TA1535: his G 46; rfa^-; $uvrB^-$:	base-pair substitutions
TA 100: his G 46; rfa^-; $uvrB^-$;R-factor:	" "
TA 102: his G 428; rfa^-; $uvrB^+$;R-factor:	" "

Regular checking of the properties of the strains regarding the membrane permeability, ampicillin- and tetracycline-resistance as well as spontaneous mutation rates is performed in the laboratory of RCC Cytotest Cell Research according to Ames et al. (1). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strains TA 1535, TA 98, and TA 100, and TA 102 were obtained from Ames (University of California, 94720 Berkeley, U.S.A.). The bacterial strain TA 1537 was obtained from BASF (D-67063 Ludwigshafen).

Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

Precultures

From the thawed ampoules of the strains 0.5 ml bacterial suspension was transferred into 250 ml Erlenmeyer flasks containing 20 ml nutrient medium. A solution of 20 µl ampicillin (25 µg/ml) was added to the strains TA 98, TA 100, and TA 102. Additionally 20 µl tetracycline (2 µg/ml) was added to strain TA 102. This nutrient medium contains per litre:

8 g Merck Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial culture was incubated in a shaking water bath for 8 hours at 37° C.

Selective Agar

The plates with the minimal agar were obtained from E. Merck, D-64293 Darmstadt.

Overlay Agar

The overlay agar contains per litre:

6.0 g MERCK Agar Agar*
6.0 g NaCl*
10.5 mg L-Histidine x HCl x H_2O*
12.2 mg Biotin*

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121° C in an autoclave.

Mammalian Microsomal Fraction S9 Mix

The bacteria used in these assays do not possess the enzyme systems, which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

S9 (Preparation by R C C - C C R)

The S9 liver microsomal fraction was obtained from the livers of 8 - 12 weeks old male rats, strain Wistar HanIbm (BRL, CH-4414 Füllinsdorf; weight approx. 220 - 320 g) which received daily applications of 80 mg/kg b.w. Phenobarbital i.p. dissolved in deionised water (Desitin; D-22335 Hamburg) and β-Naphthoflavone orally dissolved in corn oil (Aldrich, D-89555 Steinheim) on three subsequent days. The livers were prepared 24 hours after the last treatment.

After cervical dislocation the livers of the animals were removed, washed in 150 mM KCl and homogenised. The homogenate was diluted 1+3 in KCl and centrifuged at 9,000 g for 10 minutes at 4° C. A stock of the supernatant containing the microsomes was frozen in ampoules and stored at -80° C. Small numbers of the ampoules are kept at -20° C for up to one week before use. The protein content was determined using an analysis kit of Bio-Rad Laboratories, D-80939 München (Bio-Rad protein assay, Catalogue No. 5000006).

The protein concentration in the S9 preparation was 25.5 mg/ml (lot no. 050298).

S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 15% v/v in the cultures. The composition of the co-factor solution was chosen to yield the following concentrations in the S9 mix:

8 mM	$MgCl_2$
33 mM	KCl
5 mM	Glucose-6-phosphate
5 mM	NADP

in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(2).

Pre-Experiment for Toxicity

To evaluate the toxicity of the test article a pre-experiment was performed with strains TA 98 and TA 100. Eight concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described for the experiment I below (plate incorporation test).

Toxicity of the test article can be evident as a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

Dose Selection

Based upon the results of the pre-experiment the concentrations applied in the main experiments were chosen.

The maximum concentration was 5000 µg/plate. The concentration range included two logarithmic decades. In this study six adequately spaced concentrations were tested. Two independent experiments were performed.

As the results of the pre-experiment were in accordance with the criteria described below (EVALUATION OF RESULTS), these data are reported as a part of the main experiment I.

According to the dose selection criteria the test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate

Experimental Performance

For each strain and dose level, including the controls three plates were used.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µl	Test solution at each dose level, solvent (negative control) or reference mutagen solution (positive control),
500 µl	S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µl	Bacteria suspension (cf. test system, pre-culture of the strains),
2000µl	Overlay agar

In the pre-incubation assay 100 µl test solution, 500 µl S9 mix / S9 mix substitution buffer and 100 µl bacterial suspension were mixed in a test tube and incubated at 37°C for 60 minutes. After pre-incubation 2.0 ml overlay agar (45° C) was added to each tube. The mixture was poured on minimal agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.

Data Recording

The colonies were counted using the AUTOCOUNT (Artek Systems Corporation, BIOSYS GmbH, D-61184 Karben). The counter was connected to an IBM AT compatible PC with printer which printed out both, the individual and mean values of the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results).

Acceptability of the Assay

The Salmonella typhimurium reverse mutation assay is considered acceptable if it meets the following criteria:

- normal background growth in the negative and solvent control
- normal range of spontaneous reversion rates in the negative and solvent control
- the positive control substances should produce a significant increase in mutant colony frequencies

Evaluation of Results

A test article is considered positive if either a reproducible dose related increase in the number of revertants or a biologically relevant and reproducible increase for at least one test concentration is induced.

A test article producing neither a reproducible dose related increase in the number of revertants nor a biologically relevant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

A biologically relevant response is described as follows:

A test article is considered mutagenic if the number of reversions is at least twice the spontaneous reversion rate in strains TA 98, TA 100, and TA 102 or thrice on TA 1535 and TA 1537 (3, 4).

Also, a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose induced the criteria described above or not.

Range of spontaneous reversion frequencies* (3)				
1535	1537	98	100	102
10 - 29	5 - 28	15 - 57	77 - 189	121 - 293

Biometry

A statistical analysis of the data is not required.

* These results are referring to the negative control group without metabolic activation and represent our historical control range since 1993

DISCUSSION OF RESULTS

The test article Wacker BS 1701 was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100, and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls, were tested in triplicate. The test article was tested at the following concentrations:

33, 100; 333; 1000; 2500; and 5000 µg/plate

No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation.

The plates incubated with the test article showed normal background growth up to 5000 µg/plate with and without S9 mix in all strains used.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with Wacker BS 1701 at any concentration level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase in induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

REFERENCES

1. Ames, B.N., Maron D.M. (1983)
Revised methods for the Salmonella mutagenicity test
Mutation Res. 113, 173-215

2. Ames, B.N., J. McCann, and E. Yamasaki (1977)
Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test
In: B.J. Kilbey et al. (Eds.)"Handbook of Mutagenicity Test Procedures" Elsevier, Amsterdam, 1-17

3. de Serres F.J. and M.D. Shelby (1979)
Recommendations on data production and analysis using the Salmonella/microsome mutagenicity assay
Mutation Res. 64, 159-165

4. Hollstein,M., J. McCann, F.A. Angelosanto and W.W. Nichols (1979)
Short-term tests for carcinogens and mutagens
Mutation Res. 65, 133-226

Distribution of the Report

Sponsor	2x (1x original, 1x copy)
Study Director	1x (copy)

ANNEXE: TABLES OF RESULTS

Pre-Experiment for Toxicity

To evaluate the toxicity of the test article a pre-study was performed with strains TA 98 and TA 100. The results are given in the following table:

Table 1:

Substance	Concentration per plate µg	Revertants per plate TA 98		TA 100	
		–	+	–	+
Negative control	–	21	41	137	148
Solvent control	–	23	26	117	137
4-NOPD	10.0	609	/	/	/
Sodium azide	10.0	/	/	982	/
2-aminoanthracene	2.5	/	286	/	405
test article	3	20	30	107	124
	10	23	33	94	106
	33	19	36	108	126
	100	23	30	114	113
	333	20	30	111	116
	1000	28	36	117	153
	2500	23	24	117	147
	5000	16	34	118	156

– = without S9 mix
+ = with S9 mix
/ = not performed

The plates with the test article showed normal background growth up to 5000 µg/plate in strain TA 98 and TA 100.

According to the dose selection criteria, the test article was tested at the following concentrations:

33; 100; 333; 1000; 2500; and 5000 µg/plate

Experiment I: Plate Incorporation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 1535

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	11	13	13	12	1.2	
Solvent Control	15	10	19	15	4.5	1.0
Positive Control#	1014	1036	1022	1024	11.1	69.8
33	14	21	24	20	5.1	1.3
100	17	16	23	19	3.8	1.3
333	20	24	24	23	2.3	1.5
1000	18	16	15	16	1.5	1.1
2500	16	16	20	17	2.3	1.2
5000	14	13	16	14	1.5	1.0

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	5	14	9	9	4.5	
Solvent Control	12	9	10	10	1.5	1.0
Positive Control##	168	149	124	147	22.1	14.2
33	9	16	18	14	4.7	1.4
100	8	16	8	11	4.6	1.0
333	7	11	16	11	4.5	1.1
1000	19	18	16	18	1.5	1.7
2500	16	16	10	14	3.5	1.4
5000	19	11	15	15	4.0	1.5

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 1537

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	20	15	20	18	2.9	
Solvent Control	16	11	16	14	2.9	1.0
Positive Control[#]	169	140	149	153	14.8	10.7
33	10	19	16	15	4.6	1.0
100	14	11	12	12	1.5	0.9
333	10	11	17	13	3.8	0.9
1000	14	10	16	13	3.1	0.9
2500	15	7	14	12	4.4	0.8
5000	10	18	12	13	4.2	0.9

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	15	12	15	14	1.7	
Solvent Control	14	12	16	14	2.0	1.0
Positive Control[##]	122	112	101	112	10.5	8.0
33	18	20	13	17	3.6	1.2
100	9	13	15	12	3.1	0.9
333	11	12	17	13	3.2	1.0
1000	15	17	13	15	2.0	1.1
2500	8	14	16	13	4.2	0.9
5000	17	14	13	15	2.1	1.0

$$* \text{ enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

[#] 4-nitro-o-phenylene-diamine 50 µg/plate

[##] 2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 98

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	24	23	15	21	4.9	
Solvent Control	26	17	27	23	5.5	1.0
Positive Control#	614	613	601	609	7.2	26.1
33	21	21	15	19	3.5	0.8
100	21	30	17	23	6.7	1.0
333	15	19	25	20	5.0	0.8
1000	28	25	31	28	3.0	1.2
2500	21	29	20	23	4.9	1.0
5000	18	16	13	16	2.5	0.7

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	42	37	45	41	4.0	
Solvent Control	22	33	23	26	6.1	1.0
Positive Control##	316	274	268	286	26.2	11.0
33	31	36	41	36	5.0	1.4
100	29	32	29	30	1.7	1.2
333	24	29	38	30	7.1	1.2
1000	45	32	31	36	7.8	1.4
2500	22	20	31	24	5.9	0.9
5000	26	34	43	34	8.5	1.3

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# 4-nitro-o-phenylene-diamine 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 100

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	130	140	140	137	5.8	
Solvent Control	118	118	115	117	1.7	1.0
Positive Control#	955	966	1024	982	37.1	8.4
33	116	113	95	108	11.4	0.9
100	129	106	106	114	13.3	1.0
333	115	109	110	111	3.2	1.0
1000	115	111	126	117	7.8	1.0
2500	113	116	123	117	5.1	1.0
5000	131	107	117	118	12.1	1.0

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	135	158	150	148	11.7	
Solvent Control	129	139	144	137	7.6	1.0
Positive Control##	403	431	381	405	25.1	2.9
33	121	129	128	126	4.4	0.9
100	124	114	100	113	12.1	0.8
333	136	107	104	116	17.7	0.8
1000	160	151	147	153	6.7	1.1
2500	162	155	125	147	19.7	1.1
5000	156	158	153	156	2.5	1.1

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment I: Plate Incorporation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 102

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	169	220	186	192	26.0	
Solvent Control	168	197	170	178	16.2	1.0
Positive Control#	958	1023	969	983	34.8	5.5
33	190	150	167	169	20.1	0.9
100	158	166	166	163	4.6	0.9
333	152	155	167	158	7.9	0.9
1000	187	159	161	169	15.6	0.9
2500	182	164	136	161	23.2	0.9
5000	175	207	205	196	17.9	1.1

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	234	269	244	249	18.0	
Solvent Control	278	285	256	273	15.1	1.0
Positive Control##	1001	1172	1287	1153	143.9	4.2
33	166	228	234	209	37.6	0.8
100	226	221	172	206	29.8	0.8
333	196	216	268	227	37.2	0.8
1000	230	220	252	234	16.4	0.9
2500	221	208	177	202	22.6	0.7
5000	301	274	265	280	18.7	1.0

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# methyl methane sulfonate 5 µl/plate

\## 2-aminoanthracene 10 µg/plate

Experiment II: Pre-Incubation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 1535

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	28	28	23	26	2.9	
Solvent Control	29	27	30	29	1.5	1.0
Positive Control#	849	866	826	847	20.1	29.5
33	16	26	25	22	5.5	0.8
100	19	17	26	21	4.7	0.7
333	30	32	3	22	16.2	0.8
1000	22	16	23	20	3.8	0.7
2500	23	20	19	21	2.1	0.7
5000	27	13	10	17	9.1	0.6

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	14	18	15	16	2.1	
Solvent Control	13	14	13	13	0.6	1.0
Positive Control##	116	123	124	121	4.4	9.1
33	18	10	25	18	7.5	1.3
100	22	14	12	16	5.3	1.2
333	24	9	15	16	7.5	1.2
1000	20	11	22	18	5.9	1.3
2500	7	13	14	11	3.8	0.9
5000	15	16	20	17	2.6	1.3

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 1537

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	8	18	6	11	6.4	
Solvent Control	13	8	11	11	2.5	1.0
Positive Control#	134	111	139	128	14.9	12.0
33	9	12	9	10	1.7	0.9
100	7	10	10	9	1.7	0.8
333	8	7	7	7	0.6	0.7
1000	9	13	6	9	3.5	0.9
2500	11	11	6	9	2.9	0.9
5000	4	6	8	6	2.0	0.6

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	27	26	23	25	2.1	
Solvent Control	17	29	21	22	6.1	1.0
Positive Control##	142	138	97	126	24.9	5.6
33	19	32	20	24	7.2	1.1
100	17	34	9	20	12.8	0.9
333	29	22	19	23	5.1	1.0
1000	17	19	17	18	1.2	0.8
2500	18	10	20	16	5.3	0.7
5000	24	12	18	18	6.0	0.8

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# 4-nitro-o-phenylene-diamine 50 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 98

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	32	28	28	29	2.3	
Solvent Control	26	25	23	25	1.5	1.0
Positive Control#	631	641	618	630	11.5	25.5
33	28	37	29	31	4.9	1.3
100	42	31	18	30	12.0	1.2
333	31	25	32	29	3.8	1.2
1000	34	23	32	30	5.9	1.2
2500	29	32	34	32	2.5	1.3
5000	30	33	20	28	6.8	1.1

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	55	50	50	52	2.9	
Solvent Control	52	45	51	49	3.8	1.0
Positive Control##	284	254	264	267	15.3	5.4
33	47	56	60	54	6.7	1.1
100	59	42	53	51	8.6	1.0
333	34	58	54	49	12.9	1.0
1000	53	43	50	49	5.1	1.0
2500	45	45	39	43	3.5	0.9
5000	47	54	51	51	3.5	1.0

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# 4-nitro-o-phenylene-diamine 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 100

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	185	146	147	159	22.2	
Solvent Control	148	153	140	147	6.6	1.0
Positive Control#	1283	1210	1216	1236	40.5	8.4
33	138	150	130	139	10.1	0.9
100	139	146	127	137	9.6	0.9
333	134	108	123	122	13.1	0.8
1000	143	119	155	139	18.3	0.9
2500	126	119	138	128	9.6	0.9
5000	141	122	138	134	10.2	0.9

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	144	151	172	156	14.6	
Solvent Control	166	153	178	166	12.5	1.0
Positive Control##	630	722	712	688	50.5	4.2
33	171	151	185	169	17.1	1.0
100	171	156	167	165	7.8	1.0
333	165	141	173	160	16.7	1.0
1000	171	165	163	166	4.2	1.0
2500	174	163	187	175	12.0	1.1
5000	152	153	167	157	8.4	0.9

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# sodium azide 10 µg/plate

\## 2-aminoanthracene 2.5 µg/plate

Experiment II: Pre-Incubation Test

Test article: Wacker BS 1701

S9 mix from : Rat liver (Batch R 050298)

Test strain: TA 102

without S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	271	284	283	279	7.2	
Solvent Control	290	293	272	285	11.4	1.0
Positive Control#	1360	1455	1672	1496	159.9	5.2
33	227	264	220	237	23.6	0.8
100	287	258	279	275	15.0	1.0
333	232	235	215	227	10.8	0.8
1000	230	219	216	222	7.4	0.8
2500	261	218	230	236	22.2	0.8
5000	239	227	228	231	6.7	0.8

with S9 mix

Concentration µg/plate	Plate 1	2	3	Revertants / plate mean	s.d.	factor*
Negative Control	255	246	235	245	10.0	
Solvent Control	242	263	249	251	10.7	1.0
Positive Control##	956	892	1299	1049	218.9	4.2
33	226	263	203	231	30.3	0.9
100	222	269	272	254	28.0	1.0
333	248	276	251	258	15.4	1.0
1000	225	258	272	252	24.1	1.0
2500	271	261	296	276	18.0	1.1
5000	262	241	226	243	18.1	1.0

$$\text{* enhancement factor} = \frac{\Sigma \text{ revertants / concentr. test article}}{\Sigma \text{ revertants / solvent control}}$$

\# methyl methane sulfonate 5 µl/plate

\## 2-aminoanthracene 10 µg/plate

Summary of Results

Test article: Wacker BS 1701

S9 mix from: Rat liver (Batch R 050298)

without S9 mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative control	12	26	18	11	21	29	137	159	192	279
Solvent control	15	29	14	11	23	25	117	147	178	285
Positive control[#]	1024	847	153	128	609	630	982	1236	983	1496
33	20	22	15	10	19	31	108	139	169	237
100	19	21	12	9	23	30	114	137	163	275
333	23	22	13	7	20	29	111	122	158	227
1000	16	20	13	9	28	30	117	139	169	222
2500	17	21	12	9	23	32	117	128	161	236
5000	14	17	13	6	16	28	118	134	196	231

with S9 Mix

Concentration µg/plate	Revertants/plate mean from three plates									
	TA 1535		TA 1537		TA 98		TA 100		TA 102	
	I	II	I	II	I	II	I	II	I	II
Negative control	9	16	14	25	41	52	148	156	249	245
Solvent control	10	13	14	22	26	49	137	166	273	251
Positive control[##]	147	121	112	126	286	267	405	688	1153	1049
33	14	18	17	24	36	54	126	169	209	231
100	11	16	12	20	30	51	113	165	206	254
333	11	16	13	23	30	49	116	160	227	258
1000	18	18	15	18	36	49	153	166	234	252
2500	14	11	13	16	24	43	147	175	202	276
5000	15	17	15	18	34	51	156	157	280	243

[#] Sodium azide (10.0 µg/plate) strains TA 1535 and TA 100
4-nitro-o-phenylene-diamine strains TA 1537 (50 µg/plate) and TA 98 (10.0 µg/plate)
Methyl methane sulfonate (5 µl/plate) strain TA 102

[##] 2-aminoanthracene (2.5 µg/plate) strains TA 1535, TA 1537, TA 98, and TA 100
2-aminoanthracene (10.0 µg/plate) strain TA 102

1. General Items

Name of the new substance (IUPAC nomenclature)					
Other name			C A S number		
			Molecular weight		
Structural formula or rational formula			Appearance at ordinary temperature		
			Melting point		
		Physicochemical	Boiling point		
		properties	Vapour pressure		
Lot No. of new chemical substance tested		of the new chemical	Partition coefficient		
Purity of the new		substance	Solubility		
chemical substance tested	Wt %		Degree of	Water	
				DMSO	
			Solubility	Acetone	
				Others	
Name and concentration of impurities	Wt %				

2. Tester Strains

(1) Procurement

strain	Obtained from	Date obtained	Test date of chracteristics of used strain
TA 1535	Ames, 94720 Berkeley CA, U.S.A	January 10, 1994	January 13, 1994
TA 1537	BASF, D-67063 Ludwigshafen	November 14, 1994	November 17, 1994
TA 98	Ames, 94720 Berkeley CA, U.S.A	January 10, 1994	January 13, 1994
TA 100	Ames, 94720 Berkeley CA, U.S.A.	" "	" "
TA 102	Ames, 94720 Berkeley CA, U.S.A	" "	" "

(2) Preservation

Method of preservation	1. Fraction Freeze o 2. Bulk freeze ø 3. Others ( )
Storage temperature	Composition
- 196 °C	Bacterial suspension 20.0 ml
	DMSO 1.0 ml
	Others / ml

3. S9 Mix

(1) Procurement of S9

	1. Made in house ø 2. Purchase o (supplier)		
Date of preparation	February 05, 1998		
Lot No. if purchased			
Storage temperature	- 80 °C	Name and model of storage apparatus	GFL Type 6475

(2) Preparation of S9

Animals used		Inducing substance		
Species, Strain	Wistar Hanlbm	Name	phenobarbital	ß-naphtoflavone
Sex	male	Administration method	i. p.	oral
Age (in weeks)	8-12 weeks	Administration period	daily on three subsequent days	
Weight	220-320 g	(g/kg-weight)	3 x 80 mg/kg b. w.	

(3) Composition of S9 Mix

Constituents	Amount in 1 ml S9 Mix	Constituents	Amount in 1 ml S9 Mix
S9	0.15 ml	NADPH	/ µmol
$MgCl_2$	8 µmol	NADH	/ µmol
KCl	33 µmol	Na-phosphate buffer	100 µmol
Glucose-6-phosphate	5 µmol		
Glucose-6-phosphate dehydrogenase	/ µmol	Others: NADP	5 µmol

4. Preparation of the Solutions of the Positive Control Substances

	Name of substance	Supplier	Catalogue No	Grade	Purity (%)	Solvent
P	Sodium azide	Serva, D-69042 Heidelberg	30175	Research	≥ 99	deionised water
O	4-Nitro-o-phenylene-diamine	Sigma, D-82041 Deisenhofen	N 9504	Research	> 99.9	DMSO
S.	Methyl methane sulfonate	Merck-Schuchardt, D-85662 Hohenbrunn	820775	Research	> 99	deionised water
C	2-Aminoanthracene	Sigma, D-82041 Deisenhofen	A 1381	Research	97.5	DMSO
O						
N						
T.						
S	DMSO	Merck, D-64293 Darmstadt	N 16743	pure	> 99	
O						
L.						
Preparation and preservation of solutions of pos. contr.		1. Prepared when used o 2. Fraction preserved (Temperature of preservation - 20 °C) ø 3. Others ( )				

5. Preparation of test substance

Solvent used	Name	Supplier	Lot. No	Grade	Purity (%)
	DMSO	Merck, D-64293 Darmstadt	K23075631	pure	> 99

Stability of test substance in solvent used	24 hours
Reasons for selection of solvent used	better solubility than other solvents
Procedure of suspension if difficult soluble sample	
Time and temperature from Preparation to usage of solution	1 Hour Minutes 20 °C
Calculated in term of purity	Yes o No ø

6. Pre-culture

(1) Condition

	Name	Supplier	Lot. No.
Nutrient Broth	Merck Nutrient Broth	Merck, D-64293 Darmstadt	56525
Period of pre-culture		8 hours	
Time and temperature of preservation during from seeding strain to starting shaking cultivation		5 hours 20 °C	
Time and temperature of preservation during from ending shaking cultivation to use culture		1 hour 20 °C	
Type of model and manufacturer's name of equipment for shaking		GFL-Shaking Bath 1083; GFL D-30938 Burgwedel	
Method of shaking (Direction and frequency etc.)		longitudinal; 40 % of maximal frequency	
Vessel for cultivation (shape, model and volume etc.)		250 ml Erlenmeyer Vessel	
Volume of strain		500 µl	
Volume of culture medium		20 ml	

(2) Number of bacteria at the end of pre-cultivation

		Base pair substitution type			Frameshift type	
		TA 100	TA 1535	TA 102	TA 98	TA 1537
Number of bacteria survive (x 10^9/ml)	dose-selection	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0
	main test	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0
		0.5 - 1.0	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0	0.5 - 1.0
Measurement method		1. Calculation from O.D. values ϕ 2. Dilution method o 3. Others ( )				

7. Agar nutrient broth

(1) Top Agar

	Name	Agar Agar
Agar	Manufacturer	E. Merck
	Lot No	K 19938814

(2) Minimum glucose agar plate

	1. Made inhouse o 2. Purchased (Supplier) ø
Date of preparation	Prepared on
Lot No. if purchased	88054 (pre-experiment, experiment I) 88108 (experiment II)
Manufacturer of agar used	E. Merck, D-64293 Darmstadt

8. Sterility Test

	Bacterial growth other than those used for test	
Test substance	Yes	No ø
S9 Mix	Yes	No ø

9. Test Method

		Pre-incubation method	Plate method
Composition	Bacterial suspension	0.1 ml	0.1 ml
	Test substance solution	0.1 ml	0.1 ml
	Na-phosphate buffer	0.5 ml	0.5 ml
	S9 Mix (in case of metabolic activation method)	0.5 ml	0.5 ml
	Top agar solution	2.0 ml	2.0 ml
	Others:	/ ml	/ ml
Pre-incubation	Temperature	37 °C	/ °C
	Time	60 min	/ min
Incubation	Temperature	37 °C	37 °C
	Time	72 hours	72 hours

10. Method of counting of number of colonies

Method for Counting	1. Counted manually O 2. Counting apparatus ∅
Reasons for use together counting Method 1 and 2	
Name of Apparatus, Type of model, Manufacturer	Artek Counter; Artek System Corporation, U.S.A.
Correction of number of colonies counted	1. none o 2. area correction ø 3. miscount correction o 4. area and miscount correction o

11. Test results

(1) Test results should be reported on the attached form.

(2) Judgement of the results

Judgement	positive o	negative ø
Reason for judgement and referential matters: The test article did not induce point mutations in the genome of the strains used.		

12. Others

Testing Institution	Name	RCC Cytotest Cell Research GmbH
	Address	D-64380 Roßdorf Tel. 06154 / 80 7-0
Managment	Name	Markus Arenz
Head of Archive Unit	Name	Karlheinz Werner
Head of Quality Assurance Unit	Name	Frauke Hermann
Study Director	Name	Dr. Hans-Eric Wollny
	Period of experience	16 years
Personnels of Study	Name	Klaus Finkernagel
	Period of experience	12 years
	Name	
	Period of experience	
Test dates	from: March 24, 1998	to: May 12, 1998
Project No.	605900	

Table of Results

Experiment I

With (+) or without (-) S9 mix	Test substance concentration (µg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frame shift type	
		TA 100	TA 1535	TA 102	TA 98	TA 1537
S9 mix (-)	Solvent control	118 118 (117) 115	168 197 (178) 170	15 10 (15) 19	26 17 (23) 27	16 11 (14) 16
	33	116 113 (108) 95	190 150 (169) 167	14 21 (20) 24	21 21 (19) 15	10 19 (15) 16
	100	129 106 (114) 106	158 166 (163) 166	17 16 (19) 23	21 30 (23) 17	14 11 (12) 12
	333	115 109 (111) 110	152 155 (158) 167	20 24 (23) 24	15 19 (20) 25	10 11 (13) 17
	1000	115 111 (117) 126	187 159 (169) 161	18 16 (16) 15	28 25 (28) 31	14 10 (13) 16
	2500	113 116 (117) 123	182 164 (161) 136	16 16 (17) 20	21 29 (23) 20	15 7 (12) 14
	5000	131 107 (118) 117	175 207 (196) 205	14 13 (14) 16	18 16 (16) 13	10 18 (13) 12
S9 mix (+)	Solvent control	129 139 (137) 144	278 285 (273) 256	12 9 (10) 10	22 33 (26) 23	14 12 (14) 16
	33	121 129 (126) 128	166 228 (209) 234	9 16 (14) 18	31 36 (36) 41	18 20 (17) 13
	100	124 114 (113) 100	226 221 (206) 172	8 16 (11) 8	29 32 (30) 29	9 13 (12) 15
	333	136 107 (116) 104	196 216 (227) 268	7 11 (11) 16	24 29 (30) 38	11 12 (13) 17
	1000	160 151 (153) 147	230 220 (234) 252	19 18 (18) 16	45 32 (36) 31	15 17 (15) 13
	2500	162 155 (147) 125	221 208 (202) 177	16 16 (14) 10	22 20 (24) 31	8 14 (13) 16
	5000	156 158 (156) 153	301 274 (280) 265	19 11 (15) 15	26 34 (34) 43	17 14 (15) 13
Positive control not requiring S9 mix	Name	sodium azide	sodium azide	MMS	4-NOPD	4-NOPD
	Concentration (µg/plate)	10	10	5 µl/plate	10	10
	Number of colonies/plate	955 966 (982) 1024	958 1023 (983) 969	1014 1036 (1024) 1022	614 613 (609) 601	169 140 (153) 149
Positive control requiring S9 mix	Name	2-AA	2-AA	2-AA	2-AA	2-AA
	Concentration (µg/plate)	2.5	2.5	10	2.5	2.5
	Number of colonies/plate	403 431 (405) 381	1001 1172 (1153) 1287	168 149 (147) 124	316 274 (286) 268	122 112 (112) 101

Table of Results

Experiment II

With (+) or without (-) S9 mix	Test substance concentration (µg/plate)	Number of revertants (number of colonies/plate)				
		Base-pair substitution type			Frame shift type	
		TA 100	TA 1535	TA 102	TA 98	TA 1537
S9 mix (-)	Solvent control	148 153 (147) 140	290 293 (285) 272	29 27 (29) 30	26 25 (25) 23	13 8 (11) 11
	33	138 150 (139) 130	227 264 (237) 220	16 26 (22) 25	28 37 (31) 29	9 12 (10) 9
	100	139 146 (137) 127	287 258 (275) 279	19 17 (21) 26	42 31 (30) 18	7 10 (9) 10
	333	134 108 (122) 123	232 235 (227) 215	30 32 (22) 3	31 25 (29) 32	8 7 (7) 7
	1000	143 119 (139) 155	230 219 (222) 216	22 16 (20) 23	34 23 (30) 32	9 13 (9) 6
	2500	126 119 (128) 138	261 218 (236) 230	23 20 (21) 19	29 32 (32) 34	11 11 (9) 6
	5000	141 122 (134) 138	239 227 (231) 228	27 13 (17) 10	30 33 (28) 20	4 6 (6) 8
S9 mix (+)	Solvent control	166 153 (166) 178	242 263 (251) 249	13 14 (13) 13	52 45 (49) 51	17 29 (22) 21
	33	171 151 (169) 185	226 263 (231) 203	18 10 (18) 25	47 56 (54) 60	19 32 (24) 20
	100	171 156 (165) 167	222 269 (254) 272	22 14 (16) 12	59 42 (51) 53	17 34 (20) 9
	333	165 141 (160) 173	248 276 (258) 251	24 9 (16) 15	34 58 (49) 54	29 22 (23) 19
	1000	171 165 (166) 163	225 258 (252) 272	20 11 (18) 22	53 43 (49) 50	17 19 (18) 17
	2500	174 163 (175) 187	271 261 (276) 296	7 13 (11) 14	45 45 (43) 39	18 10 (16) 20
	5000	152 153 (157) 167	262 241 (243) 226	15 16 (17) 20	47 54 (51) 51	24 12 (18) 18
Positive control not requiring S9 mix	Name	sodium azide	sodium azide	MMS	4-NOPD	4-NOPD
	Concentration (µg/plate)	10	10	5 µl/plate	10	50
	Number of colonies/plate	1283 1210 (1236) 1216	1360 1455 (1496) 1672	849 866 (847) 826	631 641 (630) 618	134 111 (128) 139
Positive control requiring S9 mix	Name	2-AA	2-AA	2-AA	2-AA	2-AA
	Concentration (µg/plate)	2.5	2.5	10	2.5	2.5
	Number of colonies/plate	630 722 (688) 712	956 892 (1049) 1299	116 123 (121) 124	284 254 (267) 264	142 138 (126) 97